

insulin-precursor-containing chimeric protein by ultrafiltration.

68. The process of claim 67, wherein the ultrafiltration is carried out at a pH from about 8.0 to about 11.0.

5

69. The process of claim 68, wherein the ultrafiltration is carried out at a pH from about 9.0 to about 10.0.

10

70. The product of the process of claim 30.

15

71. An assay for screening an amino acid sequence for the ability to improve folding of an insulin precursor, comprising:

20

(a) changing the amino acid sequence of the first peptidyl fragment of the chimeric protein of claim 1, obtaining said chimeric protein with said changes, contacting said chimeric protein with said changes with at least one chaotropic auxiliary agent in an aqueous medium under conditions and for a time sufficient such that said chimeric protein folds correctly, and measuring the folding yield of said chimeric protein with said changes;

25

(b) obtaining the same chimeric protein used in step (a), but without any amino acid sequence changes described in step (a), contacting the chimeric protein without any amino acid sequence changes described in step (a) with at least one chaotropic auxiliary agent in an aqueous medium under the same conditions and for a same time used in step

(a), and measuring the folding yield of the chimeric protein; and

(c) comparing the folding yield of the chimeric proteins measured in step (a) and (b), respectively,

in which the yield measured in step (a) substantially equals or is greater than the yield measured in step (b) indicates that the amino acid sequence improves folding of the insulin

30 precursor.

72. The assay of claim 71, wherein the chimeric protein consists of the amino acid sequence of SEQ ID NO:6.

35

73. The assay of claim 71, wherein the chimeric protein consists of the amino acid sequence of SEQ ID NO:7.

74. The assay of claim 71, wherein the chaotropic auxiliary agent is urea.

5 75. The assay of claim 71, further comprising contacting the chimeric protein, in step (a) and (b) respectively, with a quantity of a mercaptan, which quantity yields less than 5 -SH radical of the mercaptan per cysteine residue of the chimeric protein.

76. The process of claim 75, wherein the mercaptan is 2-
10 mercaptoethanol.

77. The product of the assay of claim 71.

15

20

25

30

35

09/423100

Sheet 1 of 2

Figure 1A

NH2-[B1 . . . B30 . C1 . . . C35 . A1 . . . A21]-COOH

Figure 1B

